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PLAQUE FORMATION BY STRAINS OF 'RICKETTSIA RICKETTSII' AND RICK--ETC(U)
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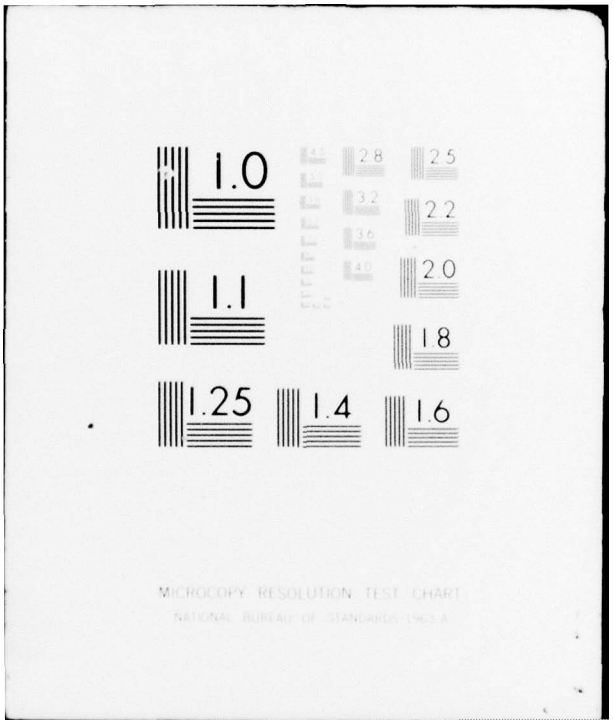
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Plaque Formation by Strains of Rickettsia rickettsii and Rickettsia parkeri in Monolayers of Various Cell Types

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ABSTRACT

Strains of Rickettsia rickettsii and Rickettsia parkeri could be differentiated by their ability to form plaques when grown in monolayers of various cell types under defined conditions.

Anong strains of Rickettsia rickettsii, the primary differentiating characteristic is virulence for the guinea pig, a property originally described in detail by Price (4). Another reproducible distinguishing characteristic such as growth in tissue culture would be useful. Present studies were undertaken to determine whether the formation of plaques or the lack of their development in a variety of cell types could be used to differentiate among these rickettsial strains.

Cell lines used were obtained from the American Type Culture Collection (ATCC); the Certified Cell Line (CCL) number is noted in Table 1. Chick embryo fibroblast (CF) cells were obtained from fertile hens' eggs supplied by SPAFAS, Inc., Norwich, Conn., and duck embryo fibroblast (DF) cells from duck eggs supplied by Truslow Farms, Chestertown, Md. Fibroblast cultures were prepared by the method of Wike et al. (7). Strains of R. rickettsii examined were: Sheila Smith (1), Bitter Root (5), R strain (1), Iowa (1), Bates (a strain isolated in 1956 by F. M. Bozeman at the Walter Reed Army Institute of Research), and Montana, ATCC VR-611. Strains of Rickettsia parkeri examined were, Strain C (6) and Maculatum (1). Seed stocks of all strains were propagated in embryonated eggs and maintained frozen at -70 C as 50% yolk sac suspensions in sucrose-phosphate-glutamate solution (2).

In experiments reported here, cell suspensions in appropriate growth media were inoculated into 25 cm^2 screw-capped plastic tissue culture flasks and allowed to form confluent monolayers. Fluid was drained from the flasks and serial dilutions of rickettsial stock cultures in brain-heart infusion broth were added (0.2 ml per flask) and distributed over the cell monolayer. After incubation at 34 C for 1 h, 5 ml Eagle's minimum essential medium containing nonessential amino acids and 0.25% agarose (SeaKem ME) were added to the cultures and allowed to solidify. The tightly capped flasks were incubated at 34 C for 7 days, then stained with 2 ml of a 1:100 dilution of neutral red dye solution (Grand Island Biological Co.) and observed for plaques. CF cultures were included in each experiment as a control to be certain of the presence of viable rickettsiae.

Results of these experiments are presented in Table 1. A number of rickettsial strains could be differentiated by plaque formation in the various cell lines. The highly virulent strains of R. rickettsii, Sheila Smith, R, and Bitter Root, formed plaques in all cell lines tested except HeLa and HEP-2 cultures and could not be distinguished one from another. The less virulent, Bates and Iowa, and the essentially avirulent Montana strains failed to form plaques in HeLa and HEP-2 cell cultures and in monolayers of one or more additional cell types. Each of these latter three rickettsial strains developed plaques in a different set of cell cultures and could be differentiated from each other and from the virulent strains. The strain C and Maculatum strains, derived from a different species of spotted fever rickettsia, R. parkeri, also formed plaques in a unique set of cell lines which was identical for these two strains, but different from those for the R. rickettsii strains.

Three morphologically different plaque types were observed in this study, the characteristics of which were due to the cell culture rather than the rickettsial strain. In most cell lines rickettsiae formed clear, round plaques varying in diameter from 0.5 to 2.0 mm (Figure 1A). In WI-38 and BHK cell lines they formed stellate plaques (Figure 1B), similar to those found by Osterman and Parr (3) for Rickettsia conorii in WI-38 cell cultures. In primary chick and duck fibroblast cultures rickettsiae developed round to slightly oval plaques measuring 1.0 to 2.0 mm in diameter. These plaques frequently had a darker staining inner ring which gave them a target-like appearance (Figure 1C). Among the cell lines tested, the BS-C-1 cultures were especially useful since most rickettsiae formed well-defined, round, clear plaques with sharp edges. This cell line was also more sensitive for a given inoculum than chick fibroblast cultures and where it could be used, it made an excellent assay system.

The rickettsial strains used in this study were all capable of multiplying at least to a limited extent in each cell line tested, whether or not plaques were formed, since organisms could be found in 7-day cultures in numbers greater than were originally added. The apparent inability of some cell lines such as HeLa or HEP-2 to allow the formation of plaques may have been due to the conditions employed. A few plaque-like areas were occasionally observed in HeLa cell cultures after long incubation; however, under the standard conditions described, plaque production was not observed.

These studies indicate that the formation or lack of development of plaques in a variety of cell cultures under defined conditions may be used to distinguish among strains of R. rickettsii. This approach

may also prove useful for differentiating among other species of spotted fever rickettsiae.

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TABLE 1. Plaque formation by rickettsial strains in various cell culture monolayers

Cell types ATCC no.	Plaque morphology[b]	Presence of plaques[a]							
		R. rickettsii						R. parkeri	
		Sheila Smith	R Strain	Bitter Root	Bates	Iowa	Montana	Maculatum	Strain C
Human									
CCL 25 (WISH)	R	+	+	+	+	+	+	+	+
CCL 23 (HEP-2)	ND	0	0	0	0	0	0	0	0
CCL 75 (WI-38)	S	+	+	+	0	+	ND	0	0
CCL 2 (HeLa)	ND	0	0	0	0	0	0	0	0
Monkey									
CCL 26 (BS-C-1)	R	+	+	+	+	0	0	+	+
CCL 81 (Vero)	R	+	+	+	+	0	ND	0	0
CCL 7 (MK-2)	R	+	+	+	+	+	0	0	0
Hamster									
CCL 10 (BHK)	S	+	+	+	0	0	+	+	+
Mouse									
CCL 1 (L-929)	R	+	+	+	+	+	+	0	0
Chick									
CF (primary)	T	+	+	+	+	+	+	+	+
Duck									
DF (primary)	T	+	+	+	+	+	+	0	ND

[a]Symbols: +, plaques formed; 0, no plaques formed; ND, no data.

[b]R, round, clear plaques; S, stellate plaques; T, target-like plaques.

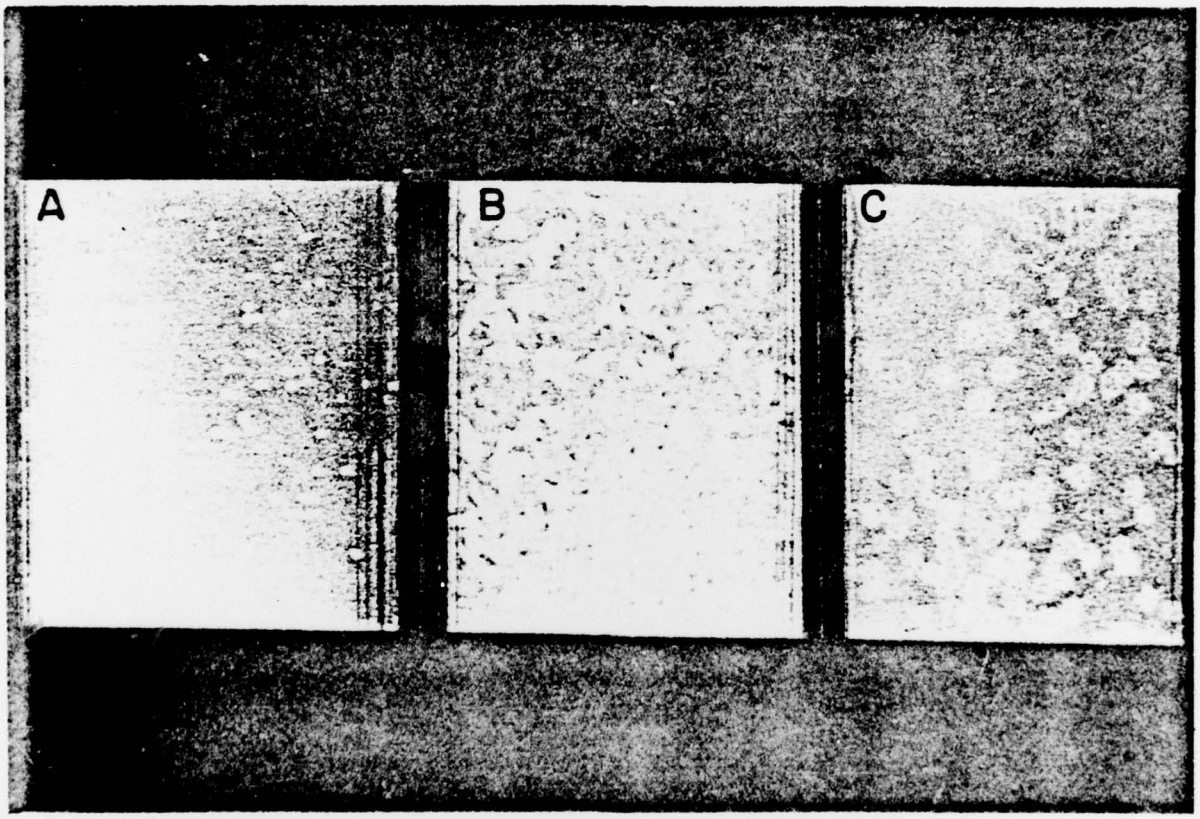
A
B
C

FIGURE LEGENDS

Figure 1. Plaque morphology developed in several tissue cell monolayers by the Bitter Root strain of R. rickettsii. (A) Round clear plaques in BS-C-1 cell cultures, (B) Stellate plaques in WI-38 cell cultures, (C) Target-like plaques in CF cell cultures.